ORIGINAL ARTICLE

Dual Specific Oral Tolerance Induction Using Interferon Gamma for IgE-Mediated Anaphylactic Food Allergy and the Dissociation of Local Skin Allergy and Systemic Oral Allergy: Tolerance or Desensitization?

G Noh,[1,2,3,4] EH Jang[4]

[1]Department of Paediatrics, Chungnam National University, Dajeon, Korea
[2]Department of Paediatrics, Pyeongtaek International Hospital, Pyeongtaek, Korea
[3]Seoul Allergy Clinic, Seoul, Korea
[4] Jeju International Allergy Centre, Hanmaum General Hospital, Jeju City, Jeju Province, Korea

Abstract

Background: Specific oral tolerance induction (SOTI) for IgE-mediated food allergy (IFA) can be successfully achieved using interferon gamma (classic SOTI).
Objective: In this study, a tolerable dose was introduced during tolerance induction with interferon gamma (dual SOTI), and its effectiveness was evaluated.
Methods: The study population comprised 25 IFA patients. Blood samples were taken for analysis, including complete blood count with differential counts of eosinophils, serum total IgE levels, and specific IgE for allergenic foods. Skin prick tests were conducted with the allergens. Oral food challenges were performed to diagnose IFA. Ten patients received dual SOTI, 5 received classic SOTI, 5 received SOTI without interferon gamma (original SOTI), and 5 were not treated (controls).
Results: Patients treated with dual SOTI and classic SOTI using interferon gamma became tolerant to the allergenic food. The tolerable dose was introduced successfully in dual SOTI. It was difficult to proceed with the same dosing protocol used for classic SOTI in cases treated with original SOTI. Following dual SOTI, the systemic reaction to oral intake subsided, but the local skin reaction to contact with the allergenic food persisted.
Conclusions: Dual SOTI is an improved protocol for SOTI using interferon gamma for IFA. The local skin reaction and systemic reaction to oral intake were dissociated following dual SOTI. In cases of food allergy, tolerance appears to result from desensitization to allergens.

Key words: Specific oral tolerance induction (SOTI). Interferon gamma. Food allergy. Atopic dermatitis. Oral immunotherapy. Desensitization. Tolerance.

Resumen

Antecedentes: La inducción de tolerancia oral específica (SOTI) para la alergia alimentaria mediada por IgE (IFA) se ha logrado empleando IFN-γ (SOTI mejorada).
Objetivo: Se evaluó la eficacia de la administración de dosis tolerables de alimento junto con IFN-γ durante la inducción de tolerancia (SOTI doble).
Material y métodos: Se incluyeron 25 pacientes con IFA. Se analizaron muestras de sangre, realizando un hemograma completo con recuento diferencial de eosinófilos, niveles de IgE total en suero, y de IgE específica, así como pruebas cutáneas para los alimentos implicados. El diagnóstico final de IFA se realizó mediante provocación oral controlada. Diez pacientes recibieron SOTI con dosis tolerables de alimento e IFN-γ (SOTI doble), 5 recibieron SOTI usando solo IFN-γ (SOTI mejorada), 5 recibieron SOTI sin IFN-γ (SOTI convencional), y 5 no fueron tratados (grupo control).

Resultados: Los pacientes tratados con SOTI doble y SOTI mejorada utilizando IFN-γ, alcanzaron la tolerancia del alimento alergénico. La dosis tolerable se alcanzó con éxito en la SOTI doble. Fue difícil el aplicar el mismo protocolo con la misma dosis en la SOTI mejorada y en los casos tratados con SOTI sin IFN-γ (SOTI convencional). Mediante SOTI doble, las reacciones sistémicas a la ingesta oral disminuyeron; sin embargo, la reacción local cutánea al contacto con el alimento alergénico permaneció invariable.
Conclusiones: La SOTI doble es un protocolo mejorado para SOTI utilizando IFN-γ para el tratamiento de la IFA. La reacción local de la piel y la reacción sistémica a la ingesta oral son independientes tras la realización de la SOTI doble. La inducción de tolerancia parece ser el resultado de la desensibilización a los alérgenos.

Palabras clave: Inducción de tolerancia oral específica (SOTI). IFN-γ. Alergia alimentaria. Dermatitis atópica. Inmunoterapia oral. Desensibilización. Tolerancia.

Introduction

Oral tolerance induction is an increasingly important topic in allergy, and attempts at generating immunotherapies for food allergens have been pursued since the 1960s [1-3]. The results of sublingual and subcutaneous administration of immunotherapeutic agents proved to be unconvincing [4,5]; however, reports of successful subcutaneous immunotherapy for allergy to fish and peanuts were published in 1997 [6,7]. Oral immunotherapy (OIT) was applied for the treatment of food allergies by Patriarca et al [8]. The term *specific oral tolerance induction* (SOTI) was coined by Noh in 2001 [9] in a report that described oral immunotherapy using interferon gamma for non–IgE-mediated food allergy in atopic dermatitis [10]. Many reports have demonstrated varying effectiveness in the use of SOTI for IgE-mediated food allergy (IFA) without interferon gamma (original SOTI) [11,12]. In original SOTI, a tolerable dose is determined following treatment using a scheduled dose. Classic SOTI, in which a tolerable dose is determined after treatment is completed, has also proven to be successful for the treatment of IFA [13]. In the present study, we added an original SOTI protocol to a classic SOTI protocol by introducing the tolerable dose during induction of tolerance with interferon gamma (dual SOTI). The effectiveness of dual SOTI was evaluated. Tolerance of and desensitization to food allergens are also discussed.

Materials and Methods

Subjects and Study Design

Participants were chosen from patients attended at the International Allergy Centre at Pyeongtaek International Hospital (Pyeongtaek, Korea), the Department of Allergy and Clinical Immunology at the Seoul Allergy Clinic (Seoul, Korea), and the Subdivision of Allergy and Clinical Immunology at the Department of Paediatrics of Chungnam National University Hospital (Daejeon, Korea) between 2010 and 2013. The study population comprised 25 patients (mean [SD] age, 6.1 [4.5] years; 14 males and 11 females) with repetitive symptoms and signs of IgE-mediated allergy, including anaphylaxis caused by food(s). Diagnoses of allergies to milk, egg, wheat, and soybean were made for all patients using an open oral food challenge (OFC). Ten patients underwent dual SOTI, 5 underwent classic SOTI, and 5 underwent original SOTI. Five patients were not treated and were used as a control group (controls). The patients were randomized by computer into 4 groups, and a case-control study was performed.

Consent forms were signed by either the patient or the parent. The forms detailed the possible results of SOTI using interferon gamma and the possibility of a medical emergency arising from acute anaphylactic reactions. This study was approved by the Ethics Committee of Chungnam National University Hospital, Daejeon, Korea. Changes in clinical severity were compared before and after OFC, and changes in laboratory results were compared before and after treatment using the Mann-Whitney test. $P<.05$ was considered significant for all comparisons.

Blood Tests and Skin Prick Tests

Blood tests included a complete blood count with differential for eosinophils, serum total IgE levels, and specific IgE for allergenic foods. These tests were conducted before and after tolerance induction. Allergen-specific IgE was measured using the UniCap method (Pharmacia & Upjohn Diagnostics AB).

Skin prick tests (SPTs) were conducted on the left forearm using commercial allergen extracts (Bencard). Histamine hydrochloride (1 mg/mL; Bencard) was used as a positive control. Physiologic saline, distilled water, and glycerol were used as negative controls. The reactions were quantified after 15 minutes, and the ratio of allergen wheal size to histamine wheal size was calculated. The minimum size of a positive reaction for allergens was 3 mm, with a minimum 3-mm wheal for histamine and no wheals for negative controls.

Oral Food Challenge and Skin Food Challenge

All 25 patients were placed on elimination diets prior to the food challenge with the suspected causative food. An open OFC was also conducted for IgE-mediated food allergy as described previously (Table 1) [13]. OFC was conducted according to the protocol, which depended on the patient's body weight. One patient presented very severe milk allergy, and the protocol for extreme cases was followed. The patients were fed the test food according to the protocol. Milk, eggs, wheat noodles, and soybean curd were purchased from a local market. Fresh milk and soybean curd were used for the OFC and treatment. Eggs and noodles were boiled in water for the OFC and treatment. The wet weights of the foods were measured, and the patients were challenged with the food according to the dose in the schedule. The food challenge

procedures started with an initial dose. If the patient did not present any symptoms after the initial dose, the following dose was administered. Doses were increased according to the protocol. Patients were checked for any possible positive reactions. If patients presented any IgE-mediated acute allergy symptoms at any stage during the challenge procedure, the test was discontinued, a diagnosis was made, and clinical severity was scored. Patients with negative reactions were excluded from the study, even if they had a clear history of food-induced anaphylaxis.

Vital signs, breath and heart sounds, skin manifestations, and itching were checked periodically during the challenge tests and the treatment. An intravenous catheter was inserted before the open challenge, and treatment and oxygen saturation were monitored. Emergency medications were prepared and kept ready to treat emergencies during the open challenge or treatment.

Confirmatory open challenge tests were also performed after completion of the SOTI using interferon gamma to confirm the acquisition of tolerance to the allergenic foods. The clinical severity score was measured after the OFC.

A skin food challenge was performed by applying raw allergenic foods to the perioral area (on the cheek) using 1 mL of milk and 1 g of eggs, wheat, or soybeans. The food was scrubbed and dispersed over the skin, and the response was examined 1 hour after challenge. Systemic reactions and local skin reactions were observed, and clinical severity was scored based on the clinical severity scoring system for IFA before and after the skin food challenge.

Diagnosis of IFA and Clinical Severity Scoring System

The diagnosis of IFA was made based on characteristic symptoms and signs as described in the clinical severity scoring system for OFC. The clinical severity of IFA was evaluated using a modification of the Clark scoring system, as previously described [14]. The symptoms and signs were classified into 4 categories: (*1*) acute allergic skin manifestations, such as urticaria and angioedema; (*2*) respiratory symptoms, such as dyspnea, wheezing, and cyanosis; (*3*) circulatory symptoms and signs of hypotension or palpitation; and/or (*4*) gastrointestinal and other symptoms. Five points were assigned for patient responses in each category. Ten points were added if the time to onset was less than 15 minutes, and 5 points were added if the time to onset was greater than 15 minutes [13]. The patients had no symptoms or signs before the OFC. If patients displayed apparent symptoms or signs matching the descriptions presented above, the diagnosis was made, and the OFC was stopped. The diagnosis of IFA was clear because of the clinical characteristics and because the symptoms and signs were absent before the OFC and only appeared after the OFC.

Oral Immunotherapy Using Interferon Gamma

Recombinant interferon gamma (Intermax γ, LGCI) with a specific activity of 2×10^6 IU (50 μg) was administered by subcutaneous injection at a dosage of 3×10^6 IU/m^2 as previously reported [14]. A scheduled dose of food was administered 10-20 minutes after patients received an

Table 1. Dose Protocols for the Open Food Challenge for IgE-Mediated Anaphylactic Food Allergy[a]

Weight ≥30 kg						Extreme case			Weight < 30 kg					
	Milk, mL	Eggs, g	Wheat, g	Soybeans, g	Observation Time		Milk, mL	Observation Time		Milk, mL	Eggs, g	Wheat, g	Soybeans, g	Observation Time
Initial						Initial			Initial					
Dose	1	1	1	1	15 min	Dose	0.001	15 min	Dose	0.1	0.1	0.1	0.1	15 min
2nd	2	2	2	2	15 min	2nd	0.01	15 min	2nd	0.2	0.2	0.2	0.2	15 min
3rd	3	3	3	3	15 min	3rd	0.05	15 min	3rd	0.5	0.5	0.5	0.5	15 min
4th	5	5	5	5	15 min	4th	0.1	15 min	4th	1	1	1	1	15 min
5th	10	10	10	10	30 min	5th	0.2	15 min	5th	2	2	2	2	30 min
6th	20	20	20	20	30 min	6th	0.5	15 min	6th	5	5	5	5	30 min
7th	50	50	50	50	1 h	7th	1	15 min	7th	10	10	10	10	1 h
8th	100		100	100	1 h	8th	2	30 min	8th	20	20	20	20	1 h
9th	200		120	120	12 h	9th	5	30 min	9th	50	50	50	50	12 h
						10th	10	1 h						
						11th	20	1 h						
						12th	50	12 h						

[a]The dose was scheduled according to the body weight (30 kg). In extreme cases, the dose was modulated.

Table 2. Protocols for Specific Oral Tolerance Induction (SOTI)[a]

Starting and Induction of Dual SOTI for Milk, Eggs, Soybean, and Wheat						
Sequence	1	2	3	4	5	Food
Extreme Case	0.01	0.1	0.2	0.5	1	Milk
Under 30 kg		0.1	0.2	0.5	1	All foods
Over 30 kg					1	All foods

Progression of Dual SOTI for Milk, Eggs, Soybean, and Wheat

- Milk, Soybeans, and Wheat

Range		10-30		30-60			60-100				
Subrange	1-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	
Incremental Dose	1	1	2	3	3	3	4	4	4	4	
Tolerable Dose		1	2	3	5	10	20	40	60	80	100

- Eggs

Range		10-30		30-50		
Subrange	1-10	10-20	20-30	30-40	40-50	
Incremental Dose	1	1	2	3	3	
Tolerable Dose		1	2	5	10-40	50

[a]SOTI was started with the initial dose. The initial dose was determined according to the minimum provocation dose by oral food challenge.

Table 3. Representative Dose Protocol for Dual SOTI for Milk and Eggs[a]

Sequence (Milk)	Treating Dose, mL	Tolerable Dose, mL	Sequence (Milk)	Treating Dose, mL	Tolerable Dose, mL	Sequence (Eggs)	Treating Dose, g	Tolerable Dose, g	Sequence (Eggs)	Treating Dose, g	Tolerable Dose, g
1	0.01		30	33	3	1	1		21	22	2
2	0.1		31	36	3	2	2		22	24	2
3	0.2		32	39	3	3	3		23	26	2
4	0.5		33	42	5	4	4		24	28	2
5	1		34	45	5	5	5		25	30	5
6	2		35	48	5	6	6		26	33	5
7	3		36	51	10	7	7		27	36	5
8	4		37	54	10	8	8			39	5
9	5		38	57	10	9	9		29	42	10
10	6		39	60	20	10	10	1	30	45	20
11	7		40	64	20	11	11	1			
12	8		41	68	20	12	12	1			
13	9		42	72	40	13	13	1			
14	10		43	76	40	14	14	1			
15	11		44	80	40	15	15	1			
16	12		45	84	60	16	16	1			
17	13		46	88	60	17	17	1			
18	14		47	92	80	18	18	1			
19	15		48	96	80	19	19	1			
20	16		49	100	80	20	20	1			
21	17	1	50		100						
22	18	1									
23	19	1									
24	20	1									
25	22	2									
26	24	2									
27	26	2									
28	28	2									
29	30	3									

[a]Wheat and soybeans were compatible with the milk dose protocol. The tolerable dose was introduced after successful treatment with 10 mL of milk (arrows).

injection of interferon gamma. Doses other than the treatment dose were absolutely prohibited and not used until treatment was finished.

For the classic SOTI protocol, food intake was increased gradually every day from a starting dose to a target dose, and interferon gamma was administered before any dose of allergenic food during treatment. The initial dose was determined according to weight and the minimum provocation dose (Tables 2 and 3). If the minimum provocation dose was ≤0.01 (mL or g), the initial dose was 0.01 (mL or g). In addition, if the minimum provocation dose was ≤0.1 (mL or g) but over 0.01 (mL or g), then the initial dose was 0.1 (mL or g). Otherwise, the initial dose followed the protocol by weight. If the patient had a positive reaction to the allergen, the dose was not increased, and the same dose was administered again in the next step. This process was repeated until the patient no longer had an allergic response to the allergen, when the dosage was increased as indicated in the schedule. Treatment was administered in the morning once a day, nearly every day except holidays and Sundays.

For the dual SOTI protocol, the treatment process was the nearly the same as in classic SOTI. However, the dual protocol included the simultaneous introduction of a scheduled tolerable dose during treatment. The tolerable dose was started when the patients successfully received 10 mL of milk or 10 g of eggs, soybeans, and wheat with no symptoms or signs. The tolerable dose was also increased according to the schedule with the progression of treatment. The treatment dose was administered with interferon gamma in the morning, and the tolerable dose was administered without interferon gamma in the evening. Treatment was performed 3 hours after breakfast in the morning, and the tolerable dose was administered 3 hours after dinner in the evening. The original SOTI protocol, in which allergenic food was given without interferon gamma, was with the dosing protocol used in classic SOTI to enable a comparison of the effectiveness of treatment. The principle

Table 4. Patient Profiles and Clinical Results by Oral Food Challenge

Patient Profile								OFC Profile (Minimum Provocation Dose and Clinical Symptoms and Signs)							Treatment		
Patient No.	Sex	Age, y	Food	ER Hx	Syconpe Hx	sIgE	SPT	Minimum Provocation Dose	Skin		Symptoms and Signs				Tx	Initial Dose	Unit
											Angioedema	Respiratory					
1	M	5	Milk	3		13.5	4	0.01	Rash	Urticaria	Angioedema	Cough	Dyspnea	Wheeze	Du	0.01	mL
2	M	3	Milk	2		9.5	3	0.1	Rash	Urticaria	Angioedema	Cough	Dyspnea	Wheeze	Du	0.1	mL
3	F	3	Milk	1		19.5	2.5	0.1	Rash	Urticaria		Cough			Cs	0.1	mL
4	F	13	Milk	5	Ves	24	3.5	0.5	Rash	Urticaria					N		mL
5	M	3	Milk	2		14.5	3	1	Rash	Urticaria					Du	1	mL
6	M	6	Milk	3		13	2.5	1	Rash	Urticaria					Cs	1	mL
7	M	5	Milk	2		22	2.5	1	Rash	Urticaria					Or	1	mL
8	F	3	Milk	2		17	2	2	Rash	Urticaria					Or	1	mL
9	F	2	Milk	1		14	2	2	Rash	Urticaria					Du	1	mL
10	F	2	Milk	1		19	2	2	Rash	Urticaria					N		mL
11	M	7	Eggs	5	Ves	23.1	6	0.1	Rash	Urticaria	Angioedema	Cough	Dyspnea	Wheeze	Du	0.1	g
12	M	3	Eggs	1		28	5	0.1	Rash	Urticaria	Angioedema	Cough			Cs	0.1	g
13	F	3	Eggs	1		18	4	0.5	Rash	Urticaria					Or	0.1	g
14	M	6	Eggs	4		16	7	0.5	Rash	Urticaria		Cough			N		g
15	M	18	Eggs	7	Ves	7.5	3	1	Rash	Urticaria					Du	1	g
16	F	4	Eggs	2		8	3	1	Rash	Urticaria					Cs	1	g
17	F	3	Eggs	1		7	3	2	Rash	Urticaria					Or	1	g
18	F	11	Eggs	4	Ves	15.2	2	2	Rash	Urticaria					N		g
19	M	5	Wheat	2		19	5	1	Rash	Urticaria	Angioedema	Cough			Du	1	g
20	M	8	Wheat	3		20	4	1	Rash	Urticaria					Cs	1	g
21	M	5	Wheat	2		19.3	6	2	Rash	Urticaria					Or	1	g
22	F	10	Wheat	5	Ves	15.2	4	2	Rash	Urticaria					N		g
23	M	18	Wheat	6	Ves	9.8	3	5	Rash	Urticaria					Du	1	g
24	M	3	Soy	1		15.9	4	1	Rash	Urticaria					Du	1	g
25	F	4	Soy	2		10.7	5	5	Rash	Urticaria					Du	1	g

Abbreviations: Cs (classic SOTI); Du (dual SOTI); ER Hx, number of visits to the emergency room caused by anaphylaxis; N (control); Or (original SOTI); sIgE, allergen-specific IgE levels; SPT, ratio of allergen wheal size to histamine wheal size; Syncope Hx, presence of syncope caused by anaphylaxis; Tx, treatment.

of complete tolerance to the prior treating dose was applied to all 3 treatments so that the dose was increased to the next dose only after complete resolution of the allergic reaction at the previous dosage.

Results

Clinical Description

All 25 patients displayed symptoms and signs of IgE-mediated anaphylactic food allergy and had a recent history of anaphylaxis including syncope, generalized urticaria, angioedema, and respiratory difficulties, such as choking and dyspnea. The OFC was performed for diagnosis and also to measure the minimum dose inducing allergy that required treatment. In the OFC, only the minimum provocation dose was approached. Thus, although systemic anaphylaxis and generalized anaphylaxis were not induced by the OFC, the symptoms and signs of any scoring items for anaphylactic food allergy were detected using the OFC. The minimum provocation dose and the relevant symptoms and signs during the OFC varied according to the patient and the type of food (Table 4).

Laboratory testing was performed twice before the initial OFC and after confirmative OFC following completion of treatment. The mean blood eosinophil fraction was 4.2% (2.1%), and the mean total serum IgE level was 250.3 (125.4) kU_A/L. The causative foods for IFA used in this study were milk (n=10), eggs (n=8), wheat (n=5), and soybeans (n=2). The mean allergen-specific IgE level was 16.6 (4.3) kU_A/L for milk, 15.4 (7.2) kU_A/L for eggs, 16.7 (3.8) kU_A/L for wheat, and 13.3 (2.6) kU_A/L for soybeans. The mean ratio of allergen wheal size to histamine wheal size was 2.7 (0.7) mm for milk, 4.1 (1.7) mm for eggs, 4.4 (1.1) mm for wheat, and 4.5 (0.7) mm for soybeans.

Clinical Results of Different SOTI Protocols

Dual SOTI was effective for the induction of tolerance for IFA, much in the same way as classic SOTI. Before treatment, the OFC tests revealed that patients displayed symptoms and signs of IgE-mediated anaphylactic food allergy. The clinical severity scores changed from 0.0 (0.0) to 20.5 (3.3) for patients undergoing dual SOTI, 0.0 (0.0) to 22.5 (5.3) for patients undergoing classic SOTI, and 0.0 (0.0) to 21.5 (4.5) for patients in the original SOTI group (Figure 1). However, after treatment, no patients displayed symptoms or signs, and the OFC clinical severity scores remained unchanged (0.0 [0.0] to 0.0 [0.0]) following dual SOTI using inter and following classic SOTI (0.0 [0.0] to 0.0 [0.0]). Patients receiving original SOTI were not able to endure the repetitive provocation of severe symptoms and signs, and the treatment was stopped in the initial phase (Figure 2). The clinical severity score of the control group increased from 0.0 (0.0) to 22.5 (5.3) before treatment and from 0.0 (0.0) to 22.5 (5.3) after treatment by OFC. The patients did not exhibit any reactions to the allergenic foods, and they were able to consume foods that contained the allergenic foods with negative reactions from 2 months to 3 years after finishing the treatment.

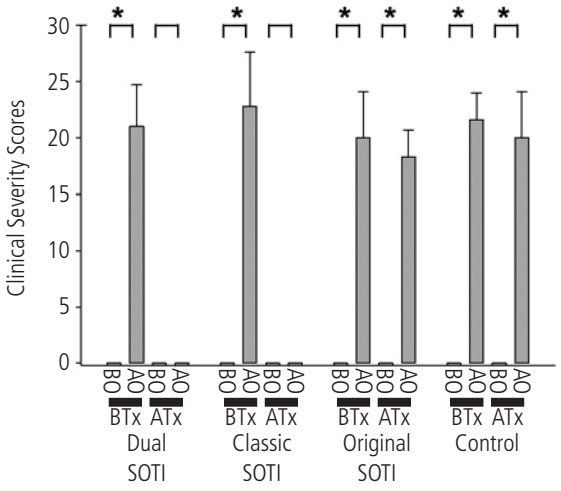

Figure 1. Dual SOTI, classic SOTI, and original SOTI protocol groups compared with the control group. SOTI indicates specific oral tolerance induction; BO, before oral food challenge; AO, after oral food challenge; BTx, before treatment; ATx, after treatment. *P<.05

Clinical Characteristics of Patients Treated with Dual SOTI

Both dual SOTI and classic SOTI were completely successful in inducing tolerance to allergenic foods. The only difference between the 2 protocols was the introduction of the tolerable dose during treatment (Figure 2). Patients did not react to the tolerable dose during treatment, even though they had shown symptoms and signs of IgE-mediated anaphylactic reactions during OFC with the same dose before treatment. Patients confirmed that they had acquired tolerance to a certain dose of the relevant food and that they could ingest the allergenic food or foodstuffs containing the allergenic food within the limits of the tolerable dose. Moreover, a tolerable dose of the allergenic food was also given to patients even during holidays or on Sundays when treatment was interrupted.

In the dual SOTI protocol, the tolerable dose was introduced successfully during treatment (Figure 3, Table 5). The tolerable dose was not given without interferon gamma. The tolerable dose did not provoke any symptoms and signs of anaphylactic IFA. Thus, it was confirmed that patients tolerated treatment at the tolerable dose. Surprisingly, the tolerable dose was higher than the minimum provocation dose of the OFC in the early phase of treatment.

Interestingly, local skin reactions to contact with the food allergens persisted despite the acquisition of systemic oral tolerance and the absence of an allergic reaction to oral intake. Although the patients achieved oral tolerance to the allergenic food upon finishing treatment, they displayed local skin reactions, such as rash and urticaria after contact with the allergenic food. However, no systemic reactions (eg, angioedema or generalized urticaria) were observed after local skin contact with the allergenic food.

Table 5. Representative Progress of Dual Specific Oral Tolerance Induction[a]

Sequence	Treating Dose, mL	Tolerable Dose, mL	Repetitive Times	Score	Sequence	Treating Dose, mL	Tolerable Dose, mL	Repetitive Times	Score
1	1		2	10	36	24	2		10
2	1			0	37	24	2		0
3	2		2	10	38	26	2		0
4	2			0	39	28	2		0
5	3			0	40	30	2		0
6	4		3	15	41	33	3		0
7	4			10	42	36	3	2	10
8	4			0	43	36	3		0
9	5			0	44	39	3		0
10	6		3	10	45	42	5		0
11	6			10	46	45	5	2	10
12	6			0	47	45	5		0
13	7			0	48	48	5	2	10
14	8		2	15	49	48	5		0
15	8			0	50	51	5		0
16	9		2	10	51	54	10		0
17	9			0	52	57	10	2	10
18	10		3	10	53	57	10		0
19	10			10	54	60	20		0
20	10			0	55	64	20		0
21	11	1		0	56	68	20	2	10
22	12	1	2	10	57	68	20		0
23	12	1		0	58	72	20		0
24	13	1	2	10	59	76	40		0
25	13	1		0	60	80	60		0
26	14	1		0	61	84	60		0
27	15	1		0	62	88	60		0
28	16	1		0	63	92	60	2	10
29	17	1		0	64	92	80		0
30	18	1	2	10	65	96	80		0
31	18	1		0	66	100	80		0
32	19	1		0	67	110	100		0
33	20	1		0	68	120	110		0
34	22	2		0	69	130	120		0
35	24	2	3	15	70		130		

[a]At a certain dose, allergy provocation occurred, and the treatment was repeated twice ( ) or even 3 times ( ) at the same dose according to the principle of overcoming the prior dose. The tolerable dose was introduced after successful treatment with 10 mL of milk (arrow).

Laboratory Changes Following SOTI Using Interferon Gamma

The blood eosinophil content did not change significantly in any group: 4.5 (1.3) to 4.2 (1.1) kU_A/L (P=.451) for the dual SOTI group; 4.3 (1.1) to 4.4 (1.3) kU_A/L (P=.451) for the classic SOTI group; 4.3 (1.2) to 4.4 (1.3) kU_A/L (P=.451) for the original SOTI group; and 4.3 (1.1) to 4.5 (1.3) kU_A/L (P=.451) for the control group. The total serum IgE levels did not change in any group: 224.2 (111.8) to 253.2 (115.3) kU_A/L for the dual SOTI group (P=.653); 213.8 (109.5) to 253.2 (115.3) kU_A/L for the classic SOTI group (P=.653); 243.2 (155.3) to 253.2 (115.3) kU_A/L for the original SOTI group (P=.653); and 222.2 (145.2) to 253.2 (115.3) kU_A/L for the control group (P=.653).

Allergen-specific IgE levels increased after treatment in the dual SOTI group (13.8 [4.5] to 20.5 [5.3] kU_A/L, n=10, P<.001) and classic SOTI group (17.7 [6.8] to 24.4 [6.1] kU_A/L, n=5, P<.001). However, no changes were observed in the original SOTI group (16.7 [5.1] to 16.0 [4.1] kU_A/L; n=5; P=.465) or the control group (17.9 [3.4] to 17.9 [3.9] kU_A/L; n=5; P=.465) (Figure 4).

Skin-prick test reactions to the allergens decreased after treatment in the dual SOTI group (3.8 [1.2] to 2.1 [0.6]; P<.001) and classic SOTI group (3.4 [1.0] to 1.8 [0.7]; P<.001). However, no changes were observed in the original SOTI group (3.5 [1.4] to 1.7 [0.4] kU_A/L, P=.465) or the control group (3.7 [1.8] to 1.9 [0.6] kU_A/L, P=.465).

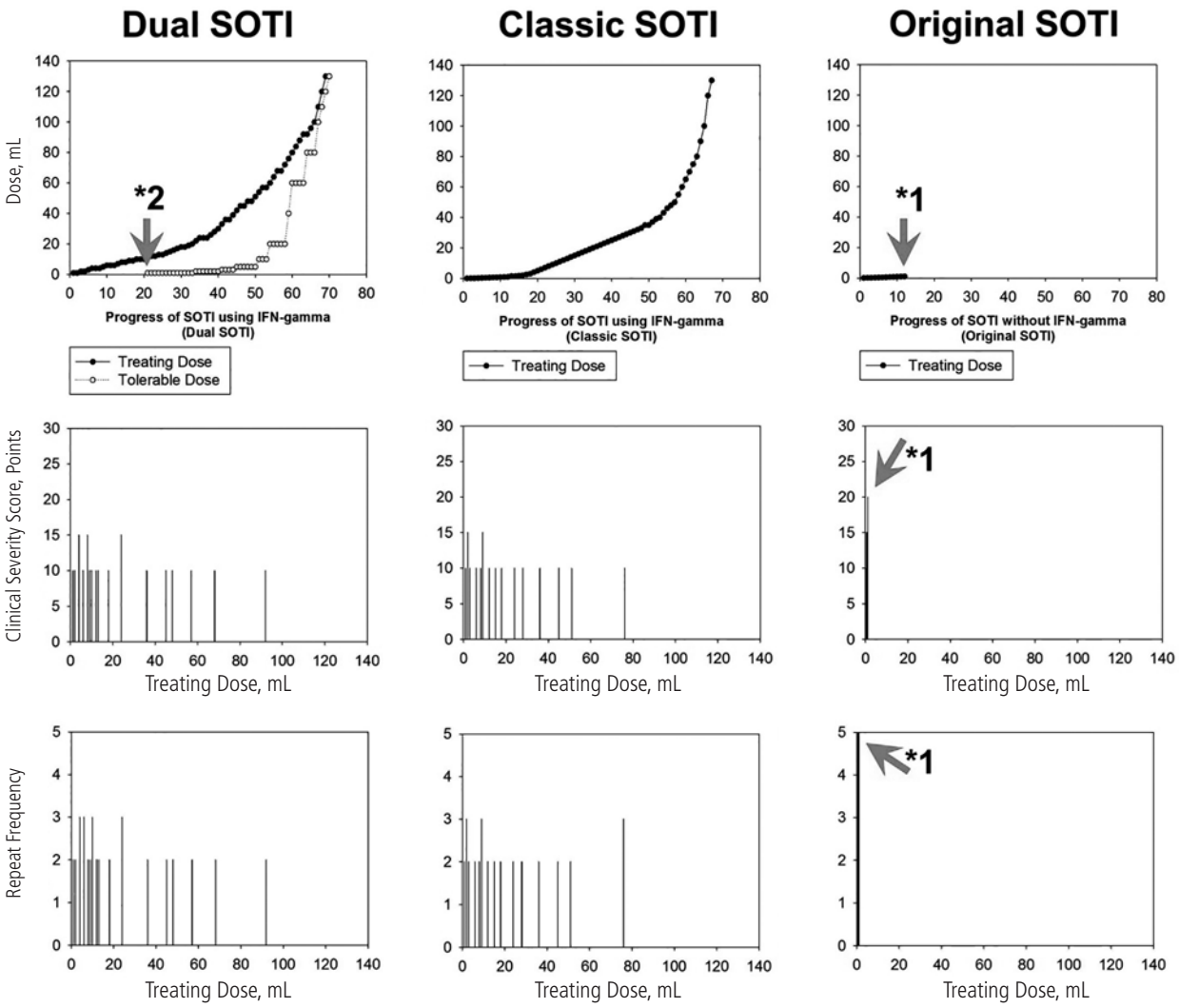

Figure 2. Clinical characteristics of dual SOTI and dose progression of dual SOTI. The treatment dose and tolerable dose were administered simultaneously during treatment. The line with the black circles represents the increment in the treatment dose, and the line with the blank circles represents the increment in the tolerable dose. The tolerable dose was increased further after the target dose was reached. The arrow (*1) indicates the cessation of treatment in the original SOTI protocol as a result of repetitive provocation of allergy. The arrow (*2) indicates the introduction of the tolerable dose during SOTI. SOTI indicates specific oral tolerance induction; IFN, interferon.

Effects of SOTI With Interferon Gamma on Skin Reactivity

Skin reactivity to the allergenic food was tested using the skin food challenge in addition to the SPT (Figure 4). In all groups, local skin reactivity, including rashes and urticaria, persisted regardless of the treatment. However, systemic reactions, including systemic urticaria, angioedema, and respiratory and cardiovascular symptoms and signs, were not observed. The clinical severity scores of the skin food challenge remained unchanged (5.0 [0.0] to 5.0 [5.0] in all groups).

Discussion

In this study, dual SOTI was an effective method of oral immunotherapy for the induction of tolerance to food allergens. A previous report on classic SOTI confirmed that patients were tolerant after administration of allergenic food even when consuming high quantities [13]. Although the only difference between the studies was that a tolerable dose was administered during the classic SOTI protocol (Figures 2 and 3, Table 5), these results are important for clinical practice and of great immunological significance. Clinicians can determine whether patients become tolerant to allergenic foods during treatment, and patients can also ingest allergenic foods at a tolerable dose during treatment. Thus, adherence to classic SOTI is high, and clinicians can administer the treatment with confidence.

The difference between dual SOTI and original SOTI stems from the use of interferon gamma as an adjuvant. In other reports, tolerance induction without interferon gamma was accomplished using an original SOTI protocol [11,12]. However, the initial dose and incremental dose were extremely low, and the treatment had to be administered over a long

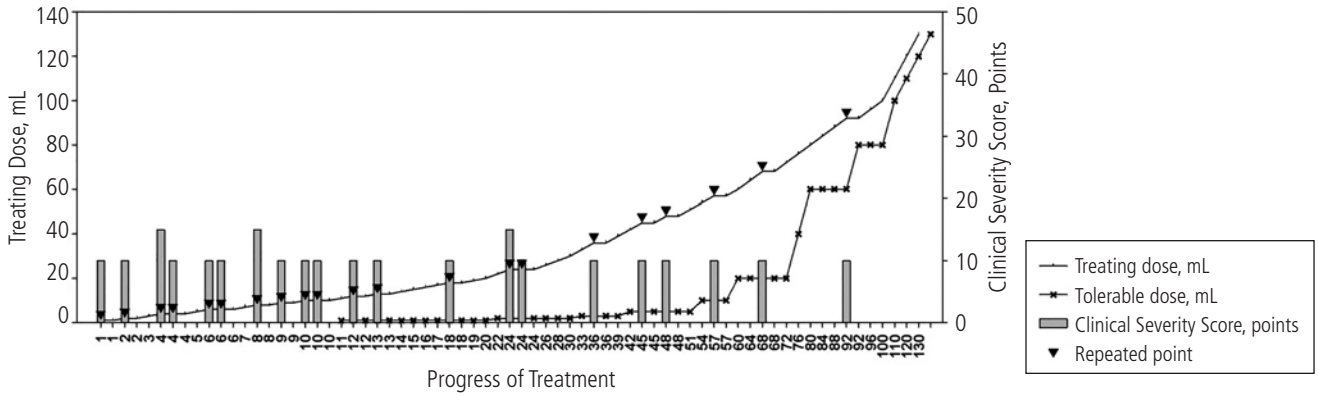

Figure 3. Representative clinical course of dual SOTI. The inverted triangles indicate the points where allergy provocation occurred during the treatment with the treatment dose, and the treatment was repeated at the next point. The bars indicate the clinical severity score at that point. The treatment dose was then repeated for doses of 1 mL, 2 mL, and other values. On some occasions, the treating dose was repeated 3 times for doses of 4, 10, and 24 mL. The X mark indicates the introduction of the tolerable dose. SOTI indicates specific oral tolerance induction.

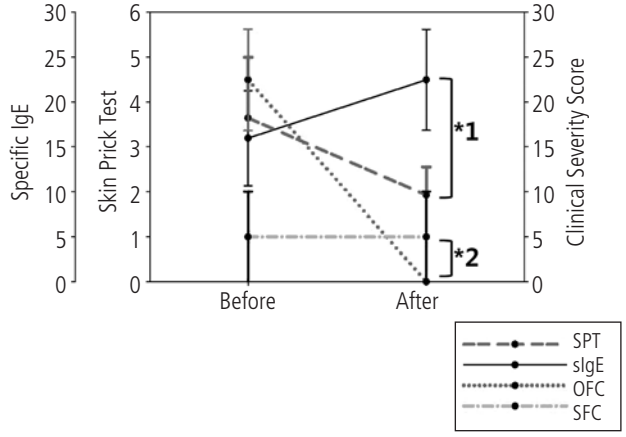

Figure 4. Dissociation between biological markers (skin prick test for allergens) and biochemical markers (allergen-specific IgE) (*1) and dissociation between systemic reaction to allergens (oral food challenge) and local skin reaction to allergens (skin food challenge) (*2). The dotted line denotes the clinical severity scores by oral food challenge; the dotted-dashed line denotes the clinical severity scores from the skin challenge test; the dashed line denotes the ratio of allergen wheal size to histamine wheal size following skin-prick tests; the solid line denotes the allergen-specific IgE.

period (as long as several years) before tolerance could be induced. Moreover, reports have shown that the effectiveness and success rate are variable. Induction of tolerance without interferon gamma appears to be difficult using our protocol, in which the initial dose and incremental dose are relatively higher than those used for the original SOTI protocol. However, by introducing interferon gamma as an adjuvant, clinicians can use a higher initial and incremental dose within a relatively short period to achieve tolerance to allergenic foods, as shown here and in a previous study [13]. In the case of allergies to house dust mite, which causes atopic dermatitis, desensitization was not successful [15], although desensitization was successful when interferon gamma was used as an adjuvant [16]. In particular, interferon gamma has been reported to be absolutely necessary for successful induction of oral tolerance in non–IgE-mediated food allergy in atopic dermatitis [17]. In the original SOTI protocol, in which interferon gamma was not used, partial induction of oral tolerance could be possible, and interferon gamma also appears to complement the tolerance induction for IFA. Based on immunological mechanisms, it is reasonable to use interferon gamma for tolerance induction for IFA. Moreover, tolerogenic effects of interferon gamma have been observed in allergen-specific regulatory B cells in vivo and in vitro [18,19]. Interferon gamma is therefore thought to be necessary for the complete effectiveness of SOTI.

The terms desensitization and tolerance can be confused in allergic disease [20]. In the original SOTI protocol, patients were fed extremely low quantities of allergenic food for desensitization. It is therefore questionable whether tolerance in the previous report of SOTI using interferon gamma was achieved in an on-off style or for gradual desensitization. However, in this report, the tolerable dose was determined according to the progress of treatment with increases in the treatment dose. Desensitization is defined as a change in the threshold dose of the ingested food allergen necessary to produce allergic symptoms; this state is dependent on ongoing antigen exposure, whereas tolerance, in contrast, is the induction of long-term immunologic changes associated with the ability to ingest a food without symptoms and without ongoing therapy [12]. Our results, on the other hand, indicate that a tolerable dose was successfully introduced, with the result that the patients were finally able to achieve tolerance as defined above, as demonstrated by their ability to consume the treated allergenic foods without any allergenic reactions from 2 months to 3 years after finishing treatment. Thus, the difference between desensitization and tolerance is not clear. Rather, in the case of dual SOTI for food allergies, desensitization is the process used to achieve tolerance, and tolerance is the result of the desensitization process. This concept was confirmed by the original SOTI protocol, in which patients received a tolerable dose using a step-by-step approach that proceeded from a low dose to higher doses without interferon gamma [11]. In dual SOTI, patients appeared to become desensitized to the allergenic food, as confirmed by the safe ingestion of the tolerable dose. However, patients displayed allergic reactions

to the higher treatment dose before they finished the treatment. Therefore, patients were allergic to the allergen at higher doses than the treatment dose yet to be administered. If patients were desensitized to the treatment dose, then they became tolerant to doses equal to or lower than those used for treatment (ie, tolerable doses); however, these patients may have still been allergic to higher doses.

Skin reactivity to allergenic foods based on the skin prick test declined after treatment, although it did not resolve completely (Figure 4), as previously reported [13,14]. Skin reactivity following the skin food challenge did not subside, even after dual SOTI and classic SOTI. In clinical practice, skin reactivity appears to decrease as treatment progresses. The symptoms and signs were localized itching, rash, or urticaria only at the area where the challenge was applied. These symptoms and signs belong to the same score category, and the clinical severity scores remained unchanged after treatment. Thus, the local skin reaction and systemic reaction following oral intake appear to be dissociated after induction of tolerance. These findings could result from immunologic differences between systemic immune reactions and the local immune reaction to the allergen. Central tolerance appears to have been induced. This type of tolerance prevents systemic allergic reactions through systemic induction of tolerogenic cells such as regulatory B cells [18,19]. However, peripheral tolerance was not induced, and local allergic reactions may have remained. This dissociation of systemic reactions and the local skin reaction provides important clues regarding sensitization to house dust mite. In particular, it may explain why house dust mite does not provoke systemic reactions, even when it produces strong reactions in SPTs and very high levels of specific IgE.

Based on the present findings, local skin responses during SOTI should be differentiated from oral allergy syndrome when advancing the treatment to the next dose. Clinicians should determine whether there was skin contact in the perioral area during the intake of allergenic foods to discriminate between local skin reaction by contact, oral allergy syndrome, and systemic allergic reactions.

In the present study, allergen-specific IgE and skin-prick tests for allergens were used only for diagnostic purposes. These tests were affected differently by the dual and classic SOTI protocols [13,14]. Unexpectedly, allergen-specific IgE levels increased after completion of dual SOTI and classic SOTI (Figure 4). This phenomenon was considered to be similar to vaccination, in which repeated allergen stimulation boosts antibody production. However, SPT results improved after dual SOTI and classic SOTI. Therefore, allergen-specific IgE is a potential biochemical marker, whereas skin reactivity to allergens following a skin prick test is considered to be a biological marker [14]. In order to provide an accurate description of IFA and tolerance induction in patients with IFA, laboratory results and clinical responses should be considered biochemical markers for allergen-specific IgE, whereas local skin reactions and systemic reactions to oral intake should be used as biological markers.

In conclusion, dual SOTI is the recommended protocol for SOTI using interferon gamma for IFA. Local skin reactions and the systemic reaction to oral intake were dissociated after dual SOTI. Food allergy tolerance appears to result from desensitization to allergens..

Acknowledgements

I would like to express special thanks to Yea Hee Yoo (Y), my wife, for her help in completing this study. I also wish to acknowledge my lovely son, Chan-Yong (Brilliant Dragon) Noh, who was born during the preparation of this manuscript, and my daughter Yeon-Seo (Researching Princess) Noh, who was born during the publication of this manuscript.

Funding

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012-0008388).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Villaveces JW. Food emulsion therapy: an adverse reaction. Ann Allergy. 1967;25:702-5.
2. Sanaka M, Hirota T, Hata M, Okuni H. Typical mil-egg albumin allergy and its desensitization therapy. Nihon Ishikai Zasshi. 1968;59:1579-86.
3. Vaillaud JC, Manassero J, Poirée M. Intolerance of beta-lactoglobulin with anaphylactic shock ameliorated by desensitization. Pediatrie. 1969l24:585-95.
4. Morris DL. Use of sublingual antigen in diagnosis and treatment of food allergy. Ann Allergy. 1969;27:289-94.
5. Podell RN. Food extract injection for food sensitivity. Valid technique or 'black magic'? Postgrad Med. 1984;76:59-62.
6. Casimir G, Cuvelier P, Allard S, Duchateau J. Life-threatening fish allergy successfully treated with immunotherapy. Pediatr Allergy Immunol. 1997;8:103-5.
7. Ison HS, Lahr J, Rule R, Bock A, Leung D. Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract. J Allergy Clin Immunol. 1997;99:744-51.
8. Patriarca C, Romano A, Venuti A, Schiavino D, Di Rienzo V, Nucera E, Pellegrino S. Oral specific hyposensitization in the management of patients allergic to food. Allergol Immunopathol (Madr). 1984;12:275-81.
9. Noh G. Food-specific oral tolerance induction for milk using interferon-gamma in atopic dermatitis. J Leukoc Biol. 2001;S:82 (Meeting Abstract: 280)
10. Noh G. Lee SS. Effects of IFN-gamma on milk-specific oral tolerance induction for milk allergy in atopic dermatitis: Food-specific oral tolerance induction using IFN-gamma as adjuvant. In: Marone G (Editor). Clinical immunology and allergy in medicine. Naples. JGC Editions; 2003. p. 475-96.
11. Staden U, Rolinck-Werninghaus C, Brewe F, Wahn U, Niggemann B, Beyer K. Specific oral tolerance induction in food allergy in children: efficacy and clinical patterns of reactions. Allergy. 2007;62:1261-9.

12. Scurlock AM, Jones SM. An update on immunotherapy for food allergy. Curr. Opin. Allergy Clin. Immunol. 2010, 10(6), 587-593.
13. Noh G, Lee SS. A pilot study of interferon-gamma-induced specific oral tolerance induction (ISOTI) for immunoglobulin E-mediated anaphylactic food allergy. J Interferon Cytokine Res. 2009;29:667-75.
14. Lee JH, Noh G, Noh J, Lee S, Choi WS, Kim HS, Lee K, Choi S, Jin H, Cho S, Lee S. Clinical characteristics of oral tolerance induction of IgE-mediated and non-IgE-mediated food allergy using interferon gamma. Allergy Asthma Proc. 2010;31:e39-e47.
15. Glover MT, Atherton DJ. A double-blind controlled trial of hyposensitization to Dermatophagoides pteronyssinus in children with atopic eczema. Clin Exp Allergy. 1992;22:440-6.
16. Noh G, Lee KY. Pilot study of IFN-gamma-induced specific hyposensitization for house dust mites in atopic dermatitis: IFN-gamma-induced immune deviation as a new therapeutic concept for atopic dermatitis. Cytokine. 2000;12:472-6.
17. Noh G, Lee JH. Oral tolerance induction for human food allergy. Inflamm Allergy Drug Targets. 2012;11:131-42.
18. Noh J, Noh G, Lee SJ, Lee JH, Kim A, Kim HS. Choi WS. Tolerogenic effects of interferon-gamma with induction of allergen-specific interleukin-10-producing regulatory B cell (Br1) changes in non-IgE-mediated food allergy. Cellular Immunology. 2012;273:140–9.
19. Lee SJ, Noh G, Lee JH. In Vitro Induction of Allergen-Specific Interleukin-10-Producing Regulatory B Cell Responses by IFN-Gamma in Non-Immunoglobulin E-Mediated Milk Allergy. Allergy Asthma Immunol Res. 2013;5(1):48-54.
20. Rachid R, Umetsu DT. Immunological mechanisms for desensitization and tolerance in food allergy. Semin Immunopathol. 2012;34:689-702.

Manuscript received December 6, 2012; accepted for publication May 7, 2013.

Geunwoong Noh

Jeju International Allergy Centre, Hanmaeum General Hospital, 52 Yeonsin-ro, Jeju city, Jeju Province, Korea 690-741
E-mail: admyth@naver.com

